Journal of Medical Virology 56:321–326 (1998)

GBV-C/HGV Coinfection in HIV-1-Positive Men: Frequent Detection of Viral RNA in Blood Plasma But Absence From Seminal Fluid Plasma

Rosalind C. Hollingsworth,[1] Claire L. Jameson,[1] Jane E. Minton,[1] Mary Crowe,[1] Rebecca Curran,[1] Tessa Rowe,[1] Anna M. Grabowska,[1] Deenan Pillay,[2] Will L. Irving,[1] and Jonathan K. Ball[1]*

[1]*Division of Microbiology and Infectious Diseases, University of Nottingham, Queen's Medical Centre, Nottingham, UK*

[2]*Public Health Laboratory Service Antiviral Susceptibility Reference Unit, Birmingham Public Health Laboratory, Birmingham Heartlands Hospital, Birmingham, UK*

Sequential paired samples of blood and seminal fluid were obtained from a cohort of 54 HIV-1-infected homosexual males. The prevalence of GBV-C/HGV RNA in the cell-free fractions of some of these patients was determined using reverse-transcription polymerase chain reaction (RT-PCR). To assess the effects of HIV-1 and HCV infection upon GBV-C/HGV RNA status, blood CD4 cell counts, HCV RNA status, and HIV-1 proviral DNA and viral RNA titres were also determined. GBV-C/HGV RNA was detected in 8/30 (27%) of the blood plasma samples obtained at the start of the study, and was present at a frequency of 14/64 (22%) in all the blood plasma samples tested. By contrast, GBV-C/HGV RNA was not detected in the 26 seminal fluid samples obtained at the start of the study, including 8 samples obtained from patients for which GBV-C/HGV RNA was detected in the corresponding blood sample. Of the samples tested for the presence of both GBV-C/HGV and HCV RNA, there was no evidence of coinfection. Although GBV-C/HGV RNA detection rates were significantly higher in individuals with blood CD4 cell counts greater than 200 cells per microlitre, there were no significant differences in the median blood CD4 cell counts or HIV-1 proviral DNA or viral RNA titres observed between the GBV-C/HGV-positive and -negative individuals. The failure to detect GBV-C/HGV RNA in seminal fluid samples obtained from this cohort would suggest that further studies need to be carried out to determine the roles of sexual transmission and of seminal fluid in GBV-C/HGV infection. ***J. Med. Virol. 56:321–326, 1998.***

KEY WORDS: GBV-C/HGV; HIV-1; seminal fluid; sexual transmission; RT-PCR

INTRODUCTION

GB virus C (GBV-C)/hepatitis G virus (HGV) is a single-stranded RNA virus and, although related to hepatitis C virus (HCV), forms a distinct group within the flaviviridae family [Linnen et al., 1996]. Similar to HCV, GBV-C/HGV demonstrates extensive genomic heterogeneity and exists as geographically-related diverse genetic subtypes [Muerhoff et al., 1996; Smith et al., 1997]. Phylogenetically related viruses have also been identified in tamarin monkeys, and these are known as GBV-A and GBV-B [Leary et al., 1996].

GBV-C/HGV infection has been associated with a variety of hepatic diseases, including non-A–E hepatitis [Linnen et al., 1996; Byrnes et al., 1996] and cholestatic liver diseases [Collambata et al., 1996; Ross et al., 1997], although an aetiological role in liver disease has not been established unequivocally [Miyakawa et al., 1997]. GBV-C/HGV infection is usually diagnosed by detecting the presence of viral RNA in blood plasma using a reverse-transcription polymerase chain reaction (RT-PCR)-based method [Dawson et al., 1996], although serological detection of antibodies to the envelope protein E2 was recently used to diagnose previous exposure to the virus [Dille et al., 1997; Tacke et al., 1997].

Epidemiological studies have shown that GBV-C/HGV is transmitted via the parenteral route and during organ transplantation [Dawson et al., 1996; Stark et al., 1996; Linnen et al., 1996; Alter et al., 1997; Moaven et al., 1997]. However, there is increasing evidence that the virus might be transmitted by hetero-

Contract grant sponsor: Medical Research Council, UK.

*Correspondence to: Dr. J.K. Ball, Division of Microbiology and Infectious Diseases, University of Nottingham, Queen's Medical Centre, Nottingham NG7 2 UH, UK. E-mail: jonathan.ball@nottingham.ac.uk

Accepted 3 June 1998

sexual [Sarrazin et al., 1997] and homosexual contact and from infected mothers to babies [Zanetti et al., 1998]. In addition, in a recent study GBV-C/HGV RNA was frequently detected in seminal fluid plasma samples obtained from patients for which both HCV and GBV-C/HGV RNA was detected in corresponding blood plasma samples, indicating that seminal fluid might serve as an important source of virus [Semprini et al., 1998]. However, to date, there have been no studies to assess the prevalence of GBV-C/HGV RNA in seminal fluid samples obtained from non-HCV-infected individuals, or from those with an increased risk of acquiring sexually transmitted diseases. To address this, we used a GBV-C/HGV-specific nested-primer RT-PCR method to test blood and seminal fluid samples obtained from a cohort of homosexual HIV-1-infected individuals. In addition, the association between HCV infection, HIV-1 virological markers and blood CD4 cell counts with GBV-C/HGV prevalence was also investigated.

MATERIALS AND METHODS

Samples

Paired samples of peripheral blood and seminal fluid were obtained from a cohort of 54 HIV-1 antibody-positive individuals, and sequential samples were obtained from 15 of these over a period of up to 12 months. Cellular and cell-free plasma fractions of blood and seminal fluid were obtained by differential and Ficoll density gradient centrifugation, respectively, and stored at −70°C until required for further use. Samples were processed within 4 hours of collection. Due to sampling limitations it was not possible to obtain data for both seminal fluid and blood from all patients, and at all time-points. Blood CD4 cell counts were determined as part of routine patient management, using FACS analysis.

Nucleic Acid Extraction

DNA was extracted from seminal fluid cells and blood lymphocytes as described previously [Ball et al., 1994; Sheehy et al., 1996], using components from a commercially available assay (Stratagene, UK). Total RNA in 50 μl of cell-free plasma was obtained using the silica absorption method included in the NASBA/Nuclisens (Organon Teknika, UK) system. Extracted nucleic acids were resupended in 50 μl of nuclease-free sterile distilled water (Sigma, UK) and stored at −70°C until required for further use.

Detection of GBV-C/HGV RNA

Eight-microlitre aliquots of extracted blood and seminal fluid plasma RNA were used as templates in reverse transcription reactions. cDNA synthesis was achieved using a commercially available cDNA synthesis kit (Pharmacia) via random hexamer primed reverse transcription in a final volume of 15 μl.

Five-microlitre aliquots of resultant cDNA was used as template in a nested primer PCR, using primers derived from the noncoding 5′ region [Jarvis et al., 1996]. First-round PCR was carried out using primer 108 (5′-AGGTGGTGGATGGGTGAT-3′, sense) with primer 531 (5′-TGCCACCCGCCCTCACCCGAA-3′, antisense), with PCR cycling parameters of 35 cycles of 94°C for 45 seconds, 50°C for 45 seconds, and 72°C for 90 seconds. One microlitre of the first-round products was then used as template in a second round, using primer 134 (5′-TGGTAGGTCGTAAATCCCGGT-3′, sense) with primer 476 (5′-GGRGCTGGGTGGCCY-CATGCW T-3′, antisense), with cycling parameters of 30 cycles of 94°C for 45 seconds, 50°C for 45 seconds, and 72°C for 90 seconds. Ten-microlitre aliquots of the second-round PCR were analysed by agarose gel electrophoresis and ethidium bromide staining.

Verification of Absence of PCR Inhibitors in Seminal -Fluid RNA Extracts

Where discordant seminal fluid and blood plasma GBV-C/HGV RNA PCR results were obtained, seven randomly chosen seminal fluid samples were tested for the presence of PCR inhibitors by carrying out spiking experiments as follows. Twenty-five-microlitre aliquots of seminal fluid were mixed with an equal volume of the corresponding blood plasma sample, and then the RNA was extracted, as described above. Further 50-μl aliquots of the blood and seminal fluid were reextracted alongside the spiked sample, and the resulting RNAs were used as templates in the RT-PCR described above. All spiked and control sample cDNAs were PCR amplified in duplicate.

Quantification of HIV-1 Viral RNA Titres

Determination of viral RNA titres in 50-μl samples of seminal fluid and blood plasma was carried out using the commercially available NASBA/Nuclisens assay system (Organon Teknika, UK). This volume was found to be the optimal amount of seminal fluid to give maximum sensitivity when using the 900-μl lysis volume. Higher inputs of seminal fluid were often associated with either assay failure or unacceptable Q_c calibrator values (results not shown). The assay was carried out according to the manufacturer's instructions, and the assay cutoff was equivalent to between 40–100 copies per 50-μl input. The higher value was taken as being the cutoff point for all samples tested, this being equivalent to 2,000 copies per millilitre.

Quantification of HIV-1 Proviral DNA Titres

Proviral DNA titres were determined using a limiting dilution nested primer PCR method designed to amplify a fragment of the HIV-1 *env* gene encompassing the hypervariable regions V1–V3. The primers used were: G1, outer, sense, 5′-GCCTGTGTACCCACACCC-CAA-3′; 401, inner, sense, 5′-GAGGATATAATCAGTT-TTATT-3′; 308, outer, antisense, 5′-ATTACAGTAGA-AAAATTCCCC-3′; and 307, inner, antisense, 5′-CT-GGGTCCCCTCCTGAGG-3′ [Simmonds et al., 1990]. PCR was carried out using primers G1 with 308 in the first round consisting of 35 cycles of 94°C for 45 seconds, 50°C for 45 seconds, and 70°C for 3 minutes. One

TABLE I. Frequency of GBV-C/HGV RNA Detection in Seminal Fluid and Blood Samples Obtained From Homosexual HIV-1-Infected Individuals

Viral nucleic acid	Sample	Cross-sectional sample detection rate (%)	Cross-sectional and longitudinal sample detection rate (%)
GBV-C/HGV RNA	Seminal fluid	0/26 (0)	0/34 (0)
	Blood	8/30 (27)	14/64 (22)

miocrolitre of this reaction was then used as template in equivalent second-round reactions using primers 401dU with 307. Proviral titres were estimated according to the Poisson formula [Simmonds et al., 1990] and calculated using the frequency of negative PCRs obtained from at least 10 replicate tubes at the end-point dilution for each sample.

Detection of HCV RNA

HCV RT-PCR was carried out using primers (0.1μM of each) derived from the 5′UTR [Garson et al., 1991]. cDNA prepared for the GBV-C/HGV-specific RT-PCR was used as template in a first-round reaction using primer PT1 (5′-CGT TAG TAT GAG TGT CGT GC-3′, sense) with primer PT2 (5′-CGG TGT ACT CAC CGG TTC C-3′, antisense) in a reaction volume of 25 μl. One microlitre of the resulting product was used as template in a similar reaction using primer PT3 (5′-AGT GTC TGT CAG CCT CCA GG-3′, sense) with primer PT4 (5′-CGG TTC CGC AGA CCA CTA TG-3′, antisense). First-round cycling parameters were 30 cycles of 94°C for 45 seconds, 50°C for 45 seconds, and 72°C for 90 seconds. Second-round cycling parameters were 30 cycles of 94°C for 45 seconds, 46°C for 45 seconds, and 72°C for 90 seconds. PCR products from the second-round reactions were analysed using ethidium bromide-stained agarose gel electrophoresis.

Statistical Analyses

All tests used were nonparametric methods. Differences in sample distributions, population medians, and detection frequencies were compared using the Kruksal-Wallis ANOVA, the Mann-Whitney U-test, and Fisher's exact test, respectively. All tests used were nonparametric because the data were not normally distributed, and were carried out using the C-Stat for Windows and Prism software packages (Chilwell Scientific, UK).

RESULTS

Detection of GBV-C/HGV RNA in Blood and Seminal-Fluid Plasma

The detection rate in cross-sectional samples obtained from a cohort of HIV-1-infected individuals is presented in Table I. Fifty-four patients were enrolled into the study, and of those samples tested, GBV-C/HGV RNA was detected in 8/30 (27%) blood-plasma samples. All of the patients who were positive for GBV-C/HGV RNA were homosexual males with no identifiable risk for parenteral transmission, including previous or ongoing intravenous drug abuse or receipt of blood or blood products. None of the 26 seminal fluid samples tested were positive for GBV-C/HGV RNA. Analysis of sequential samples obtained from some of these patients (Table II) showed that 3 patients became GBV-C/HGV RNA viraemic after the baseline sample was taken. Whilst 2 patients cleared the virus during the course of the study, one remained viraemic for the 7-month study period. Again, GBV-C/HGV RNA was not detected in any of the sequential seminal fluid samples tested (not shown), including those for which the correponding blood sample was positive. The total number of blood and seminal fluid samples tested for the presence of GBV-C/HGV RNA was 64 and 34, of which 14 (22%) and 0 (0%) were positive, respectively.

Verification of the Absence of PCR Inhibitors in Seminal-Fluid RNA Extracts

To ensure that the failure to detect GBV-C/HGV RNA in seminal fluid was not due to the presence of PCR inhibitors, samples of blood and corresponding seminal fluid from a selection of patients who were positive for GBV-C/HGV were extracted separately or as a mixture, and the resulting RNA was used as template in duplicate RT reactions. Of the 7 patients tested at random who were positive for GBV-C/HGV RNA in the blood, all the corresponding seminal fluid samples were negative, and there was no evidence of PCR inhibition in the blood samples spiked with an equal volume of seminal fluid prior to extraction. An example of the PCR products obtained from such a spiking experiment is presented in Figure 1.

Comparison of HIV-1 and HCV Nucleic Acid Detection Rates and Blood CD4 Cell Counts in Individuals With Differing Blood GBV-C/HGV RNA Status

GBV-C/HGV RNA was detected more frequently in individuals with relatively high blood CD4 cell counts. Of the 8 individuals whose baseline blood sample was positive for GBV-C/HGV RNA, only 2 (25%) had blood CD4 cell counts of less than 200 per microlitre, compared to 15/21 (71%) who were GBV-C/HGV RNA-negative ($P = 0.0382$, Fisher's exact test) (Table III). HIV-1 RNA was detected more frequently in both blood and seminal fluid samples obtained from GBV-C/HGV RNA-negative compared to GBV-/RNA-positive individuals, although this difference was not statistically significant ($P > 0.1$, Fisher's exact test). HCV RNA was detected in the blood plasma of only 1 of the 17 patients tested, and this individual was also GBV-C/HGV RNA-negative.

TABLE II. GBV-C/HGV RNA Status in Longitudinal Samples Obtained From Some of the HIV-1-Infected Homosexual Individuals Who Tested Positive for the Presence of GBV-C/HGV RNA on at Least One Occasion

Patient number	Time interval (months)	Blood GBV-C/HGV RNA status
Q15	0	+
	7	+
Q101	0	−
	2	+
	7	+
Q108	0	−
	1	+
	6	+
	12	−
Q118	0	−
	1	−
	4	+
	10	−

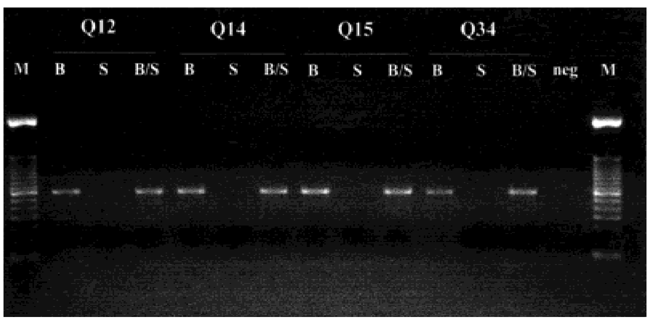

Fig. 1. Assessment of the presence of PCR inhibitors in seminal-fluid samples. Paired seminal-fluid and blood-plasma samples from patients Q12, Q14, Q15, and Q34 were extracted either separately or as an equal mixture and used as templates in RT-PCR. B, blood plasma; S, seminal fluid plasma; B/S, blood/seminal fluid mixed in equal volumes prior to extraction; neg, PCR negative control.

GBV-C/HGV RNA Detection Correlated to HIV-1 Virological and Host Parameters

HIV-1 proviral and viral RNA titres in blood and seminal fluid, together with blood CD4 cell counts, were determined to assess whether these were related to GBV-C/HGV RNA. Scatter diagrams of blood CD4 cell counts and HIV-1 proviral titres and viral RNA titres, corresponding to GBV-C/HGV RNA positive vs. negative samples, are shown in Figure 2. Comparisons of the population medians and variance (not shown) revealed that neither blood CD4 cell nor blood and seminal fluid HIV-1 viral and proviral titres varied between the GBV-C/HGV RNA-positive and -negative sample groups.

DISCUSSION

This study assessed the prevalence of GBV-C/HGV infection in a cohort of predominantly homosexual HIV-1-infected individuals. Prevalence rates within the cohort were 27%, much higher than the typical range of 1–3% reported for blood donors [Roth et al.,1997]. GBV-C/HGV RNA detection rates of 11% for homosexual and bisexual men have been reported previously [Stark et al., 1996], although no information regarding the underlying prevalence of HIV-1 infection in that study group was given. To investigate the possible effects of coinfection with HIV-1 upon GBV-C/HGV prevalence, we measured blood CD4 cell counts (as an indicator of immune suppression) and HIV-1 proviral and viral loads. The finding that GBV-C/HGV RNA was detected at higher frequency in the patients with higher blood CD4 cell counts, together with the observation that HIV-1 proviral or viral detection rate or titres were not significantly different in the GBV-C/HGV RNA-positive and -negative groups, suggests that the prevalence of GBV-C/HGV is not a significant reflection of immune suppression associated with HIV-1 infection.

Taken together, these findings indicate that sexual contact might be an important route of transmission, and GBV-C/HGV RNA detection rates of between 11–21% in cohorts of female prostitutes reported elsewhere would support this [Kao et al., 1997]. However, in that study the prevalence of HBV and HCV antibodies were present at similar frequencies to those of GBV-C/HGV RNA. Considering the very low risk of sexual transmission of HCV, these data suggest that other risk factors, such as intravenous drug abuse, might have contributed to the high prevalence of GBV-C/HGV observed in their cohort. By contrast, the detection rate of HCV RNA in 16 of the homosexual patients presented here was 1/16 (6%), compared to 5/16 (31%) who were GBV-C/HGV-positive. These disparate data support the possibility of nonparenteral transmission of GBV-C/HGV in our cohort.

To assess further the possibility of sexual transmission of GBV-C/HGV, and to identify possible reservoirs of transmissible virus, we tested seminal fluid plasma for the presence of GBV-C/HGV RNA. All of the samples tested, which included samples obtained from individuals who were GBV-C/HGV RNA-positive in their blood, were negative. This finding is in stark contrast to the recent data published by Semprini et al [1998], and there are several possible explanations as to why we failed to detect GBV-C/HGV RNA. One possibility is the presence of PCR inhibitors in the seminal fluid RNA extraction; indeed, when using a commercially available nonsilica-based extraction method, Semprini et al. [1998] reported inhibition of PCR in approximately 50% of the seminal plasma samples tested. However, to circumvent this potential problem we purposefully used nucleic acid samples generated for the HIV-1 NASBA/Nuclisens assay, which employs an extraction method that efficiently removes PCR inhibitors associated with seminal fluid nucleic acid extractions [Dyer et al., 1996], and we were consistently able to detect HIV-1 RNA from the same extracts. In addition, in a series of extractions where GBV-C/HGV-negative seminal plasma samples were mixed with GBV-C/HGV-positive blood plasma samples obtained from the same patients, there was no evidence of PCR inhibition. Alternatively, the titre of virus present in the seminal fluid of our patients might be below the

TABLE III. Comparison of Detection Frequency of HIV-1 and HCV Nucleic Acids in Blood Plasma GBV-C/HGV RNA-Positive vs. -Negative Homosexual Individuals Coinfected With HIV-1

Concurrent blood GBV-C/HGV RNA status	Proportion with AIDS[a]	Proportion GBV-C/HGV RNA-positive seminal fluid (%)	Proportion HIV-1 RNA-positive: Blood (%)	Proportion HIV-1 RNA-positive: Seminal fluid (%)	Proportion HIV-1 DNA-positive: Blood (%)	Proportion HIV-1 DNA-positive: Seminal fluid (%)	Proportion HCV RNA-positive: Blood (%)	Proportion HCV RNA-positive: Seminal fluid (%)
GBV-C/HGV-positive	2/8 (25)	0/8 (0)	4/8 (50)	4/8 (50)	15/15 (100)	7/17 (41)	0/5 (0)	0/5 (0)
GBV-C/HGV-negative	15/21 (71)	0/18 (0)	17/22 (77)	12/19 (63)	8/8 (100)	5/8 (62)	1/11 (9)	0/11 (0)

[a]AIDS was defined as individuals with blood CD4 cell counts of less than 200 per microlitre.

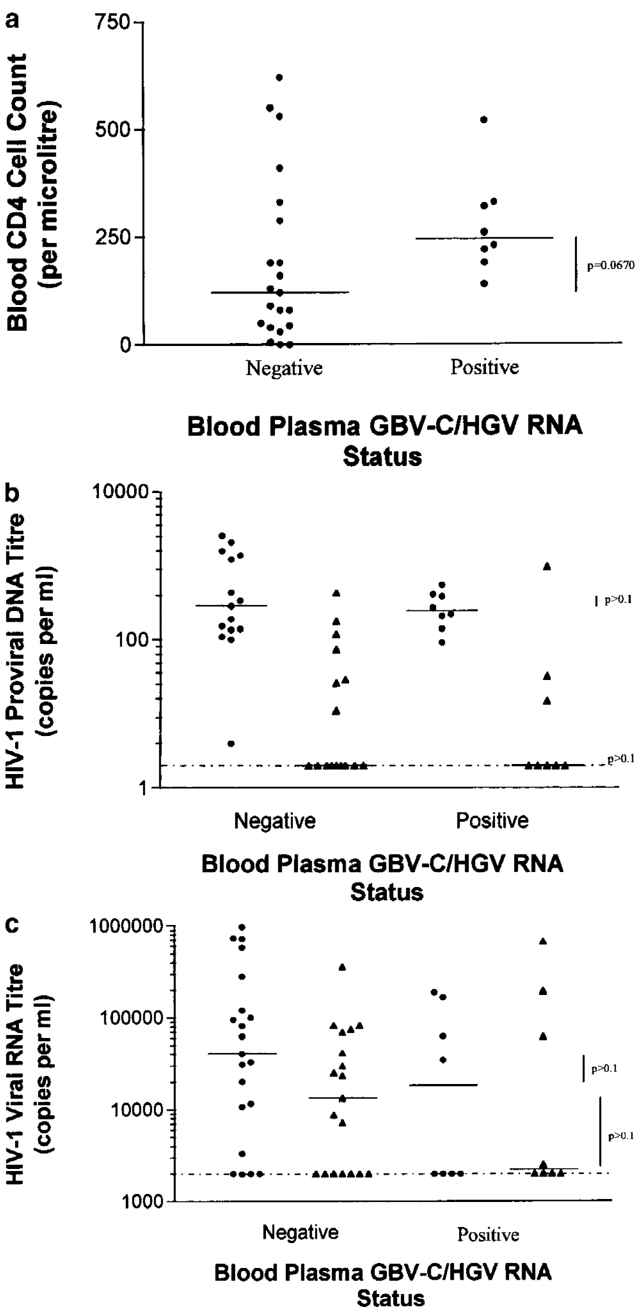

Fig. 2. Comparisons of blood CD4 cell counts **(a)** and blood (●) and seminal fluid (▲) HIV-1 proviral DNA **(b)** and viral RNA **(c)** titres in GBV-C/HGV-positive and -negative individuals. Bar represents the population median, and the Mann-Whitney U-test significance between the median values is also shown (.–.–.–.–, assay cutoff).

detection limit of our in-house RT-PCR assay, although this is unlikely as the nested primer PCR method used has a detection sensitivity of one copy of GBV-C/HGV cDNA. Another possible reason for failing to detect GBV-C/HGV RNA in the plasma is that the virus might reside in the cellular fraction of seminal fluid rather than as cell-free (plasma) virus. Seminal fluid is composed mainly of spermatazoa, immature germ cells, and leukocytes such as T cells, granulocytes, macrophages, and B cells [Wolff and Anderson, 1988]. The GBV-C/HGV RNA-minus strand, a replicative intermediate form of the viral genome, was recently detected in peripheral blood mononuclear cells [Saito et al., 1997], indicating that seminal fluid leukocytes might be an important virus reservoir. However, the studies carried out by Semprini et al. [1998] failed to detect GBV-C/HGV RNA in the cellular fraction of samples which were positive for the viral RNA in the plasma. Unfortunately, the seminal-fluid cell fractions obtained from our study group were used for HIV-1 studies which will be published elsewhere, and measurement of GBV-C/HGV RNA in these was not possible. One final possibility is that the distribution of GBV-C/HGV in the HCV-coinfected compared to HIV-1-coinfected individuals is different, although there are no obvious virological mechanisms to support this. Therefore, in conclusion, although these data suggest that sexual transmission might be an important route of GBV-C/HGV infection, further determination of the prevalence of GBV-C/HGV RNA in seminal-fluid samples obtained from a variety of cohorts needs to be carried out to determine fully the possible role of seminal fluid in this process.

ACKNOWLEDGMENTS

The authors thank Dr. Chris Bignell, Dr. I. Ahmed, Dr. C. Bowman, Dr. Mike Youle, Dr. Graeme Moyle, Alison Cargill, and Glenn Sontag for collecting the patient samples.

REFERENCES

Alter MJ, Gallagher M, Morris TT, Moyer LA, Meeks EL, Krawczynski K, Kim JP, Margolis HS (1997): Acute non-A–E hepatitis in the United States and the role of hepatitis G virus infection. New England Journal of Medicine 336:741–746.

Ball JK, Holmes EC, Whitwell H, Desselberger U (1994): Genomic variation of human immunodeficiency virus type 1 (HIV-1): Molecular analyses of HIV-1 in sequential blood samples and various organs obtained at autopsy. Journal of General Virology 75:867–879.

Byrnes JJ, Banks AT, Piatack M Jr, Kim JP (1996): Hepatitis G-associated aplastic anaemia. Lancet 348:472.

Collambata P, Randone G, Civitico G, Gorin MJ, Dolci L, Medaina N, Oliveri B, Verme G, Marchiaro G, Pagni R, Karayiannis P, Thomas HC, Hess G, Bonino F, Brunetto MR (1996): Hepatitis G virus RNA in the serum of patients with elevated gamma glutamyl transpeptidase and alkaline phosphatase: A specific liver disease. Journal of Viral Hepatitis 3:301–306.

Dawson GJ, Schlauder GG, Pilot-Matias TJ, Thiele D, Leary TP, Murphy P, Rosenblatt JE, Simons JN, Martinson FEA, Gutierrez RA, Lentino JR, Pachucki C, Muerhoff AS, Widell A, Tegtmeier G, Desai S, Mushahwar IK (1996): Prevalance studies of GB virus-C infection using reverse transcriptase-polymerase chain reaction. Journal of Medical Virology 50:97–103.

Dille BJ, Surowy TK, Gutierrez RA, Coleman PF, Knigge MF, Carrick RJ, Aach RD, Hollinger FB, Stevens CE, Barbosa LH, Nemo GJ, Mosley JW, Dawson GJ, Mushahwar IK (1997): An ELISA for the detection of antibodies to the E2 protein of GB Virus C. Journal of Infectious Diseases 175:458–461.

Dyer JR, Gilliam BL, Eron JJ Jr, Grosso L, Cohen MS, Fiscus SA (1996): Quantitation of human immunodeficiency virus type 1 RNA in cell-free seminal plasma:comparison of NASBA (TM) with Amplicor (TM) reverse transcription-PCR amplification and correlation with quantitative culture. Journal of Virological Methods 60:161–170.

Garson JA, Ring C, Tuke P (1991): Improvement of HCV genome detection with "short" PCR products. Lancet 338:1466–1467.

Jarvis LM, Davidson F, Hanley JP, Yap PL, Ludlam CA, Simmonds P (1996): Infection with hepatitis G virus among recipients of plasma products. Lancet 348:1352–1355.

Kao JH, Chen W, Chen PJ, Lai MY, Lin RY, Chen DS (1997): GB virus-C/hepatitis G virus infection in prostitutes: Possible role for sexual transmission. Journal of Medical Virology 52:381–384.

Leary TP, Muerhoff AS, Simons JN, Pilot Matias TJ, Erker JC, Chalmers ML, Schlauder GG, Dawson GJ, Desai SM, Mushahwer IK (1996): Sequence and genomic organisation of GBV-C: A novel member of the Flaviviridae associated with non-A–E hepatitis. Journal of Medical Virology 48:60–67.

Linnen J, Wages J Jr, Zhang-Keck Z-Y, Fry KE, Krawczynski KZ, Alter H, Koonin E, Gallagher M, Alter M, Hadziyannis S, Karayiannis P, Fung K, Nakatsuji Y, Shih JW-K, Young L, Piatak M Jr, Hoover C, Fernandez J, Chen S, Zou J-C, Morris T, Hyams KC, Ismay S, Lifson JD, Hess G, Foung SKH, Thoma H, Bradley D, Margolis H, Kim JP (1996): Molecular cloning and disease association of hepatitis G virus: A transfusion-transmissible agent. Science 271:505–508.

Miyakawa Y, Mayumi M (1997): Hepatitis G virus: A true hepatitis virus or an accidental tourist? New England Journal of Medicine 336:795–796.

Moaven LD, Locarnini SA, Bowden DS, Kim JP, Breschkin A, McCaw R, Yun A, Wages J Jr, Jones B, Angus P (1997): Hepatitis G virus and fulminant hepatic failure: Evidence for transfusion-related infection. Journal of Hepatology 27:613–619.

Muerhoff AS, Simns JN, Leary TP, Erker JC, Chalmers ML, Pilot-Matias TJ, Dawson GJ, Desai SM, Mushahwar IK (1996): Sequence heterogeneity within the 5′-terminal region of the hepatitis GB virus C genome and evidence for genotypes. Journal of Hepatology 25:379–384.

Ross RS, Viazov S, Kruppenbacher JP, Elsner S, Sarr S, Lange R, Eigle F-W, Roggendorf M (1997): GB virus C infection in patients who underwent liver transplantation. Liver 7:238–243.

Roth WK, Waschk D, Marx S, Tschauder S, Zeuzem S, Bialleck H, Weber H, Seifried E (1997): Prevalance of hepatitis G virus and its strain variant, the GB agent, in blood donations and their transmission to recipients. Transfusion 37:651–656.

Saito S, Tanaka K, Kondo M, Morita K, Kitamura T, Kiba T, Numata K, Sekihara H (1997): Plus- and minus-stranded hepatitis G virus RNA in liver tissue and in peripheral blood mononuclear cells. Biochemical and Biophysical Research Communications 237:288–291.

Sarrazin C, Roth WK, Zeuzem S (1997): Heterosexual transmission of GB virus-C/hepatitis G virus infection. European Journal of Gastroenterology and Hepatology 9:1117–1120.

Sheehy N, Desselberger U, Whitwell H, Ball JK (1996): Concurrent evolution of regions of the envelope and polymerase genes of human immunodeficiency virus type 1 observed during zidovudine (AZT) therapy. Journal of General Virology 76:1071–1081.

Simmonds P, Balfe P, Ludlam CA, Bishop JO, Leigh Brown AJ (1990): Human immunodeficiency virus-infected individuals contain provirus in small numbers of peripheral blood mononuclear cells and at low copy number. Journal of Virology 64:864–872.

Smith DB, Cuceanu N, Davidson F, Jarvis LM, Mokili JLK, Hamid S, Ludlam CA, Simmonds P (1997): Discrimination of hepatitis G virus/GBV-C geographical variants by analysis of the 5′ noncoding region. Journal of General Virology 78:1533–1542.

Stark K, Bienzle U, Hes G, Englel AM, Hegenscheid B, Schluter V (1996): Detection of the hepatitis G virus genome among injecting drug users, homosexual and bisexual men and blood donors. Journal of Infectious Diseases 174:1320–1323.

Tacke M, Kiyosawa K, Stark K, Schlueter V, Ofenloch-Haehnle B, Hess G, Engal A (1997): Detection of antibodies to a putative hepatitis G virus envelope protein. Lancet 349:318–320.

Wolff H, Anderson D (1988): Potential human immunodeficiency virus host cells in human semen. AIDS Research and Human Retroviruses 4:1–2.

Zanetti AR, Tanzi E, Romano L, Principi N, Zuin G, Minola E, Zapparoli B, Palmieri M, Marini A, Ghisotti D, Friedman P, Hunt J, Laffler T, Lombardy Study Group on Vertical/Perinatal Hepatitis Viruses Transmission (1998): Multicentre trial on mother-to-infant transmission of GBV-C virus. Journal of Medical Virology 54:107–112.